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(54) Title: REAGENTS AND METHODS FOR ASSESSING LEVELS OF O^6-ALKYLGUANINE-DNA ALKYLTRANSFERASE *IN VIVO*

(57) Abstract: Radiolabeled O^6-derivatized guanine molecules can be used to form radiolabeled AGT molecules which can be detected *in vivo*. *In vivo* assessment of AGT levels is useful for optimizing the efficacy of chemotherapy. In addition, assessing AGT levels in tumors *in vivo* permits selection of patients most likely to benefit from such chemotherapy and enables clinicians to monitor the effect of known and potential chemotherapeutic agents. In particular, methods have been developed for the efficient syntheses of [^{18}F]FBG and [^{131}I]IBG, both of which bind specifically to purified AGT. These two radiolabeled O^6 benzylguanine analogues can be used to assess levels of AGT *in vivo*.

REAGENTS AND METHODS FOR ASSESSING LEVELS OF O^6-ALKYLGUANINE-DNA ALKYLTRANSFERASE *IN VIVO*

This work was supported by Grants CA74817 and CA42324 from National Institutes of Health. The government therefore has certain rights in the invention.

TECHNICAL AREA OF THE INVENTION

The invention relates to the area of cancer chemotherapeutics. In particular, the invention relates to methods of assessing levels of O^6-alkylguanine-DNA alkyltransferase *in vivo*.

BACKGROUND OF THE INVENTION

A number of alkylating agents such as 1, 3-bis(2-chloroethyl)-1-nitrosourea (BCNU), 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU), 5-(dimethyltriazeno)imidazole-4-carboxamide (DTIC), and temozolomide are used in the chemotherapy of various cancers (1). The cytotoxicity of these and related alkylating agents stems from their ability to alkylate DNA guanine residues at their O^6-position. Nitrogen mustard alkylating agents such as cyclophosphamide were thought to produce their cytotoxicity through the alkylation of the O^7-position of guanine (2); however, it has been recently demonstrated that a significant part of the cytotoxicity of this class of agents also is mediated by the O^6-alkylation of guanine residues (3).

The DNA repair protein O^6-alkylguanine-DNA alkyltransferase (AGT) reverts alkylator cytotoxicity by transferring the O^6-alkyl group from the modified DNA guanine to cysteine-145 in its active site, thereby restoring a normal guanine at the site of the

modified base (4). However, the resultant alkylated AGT is inactivated for subsequent dealkylations. Because of this, the ability of tumor cells to be resistant to the toxic effects of alkylating agents is dependent on cellular AGT levels: the higher the AGT content, the less effective will be the tumor killing by alkylator therapy. For example, elevated levels of AGT are known to result in resistance of human gliomas to nitrosoureas (5). Indeed, depletion of AGT by administration of substrates such as O^6-methylguanine and O^6-benzylguanine (BG)[1] has been shown to increase the cytotoxicity of alkylators such as CCNU and BCNU in human tumor cell lines (6, 7) and xenografts (8).

Levels of AGT in human tumors vary considerably among different tumor types, as well as among patients with a particular malignancy (9, 10). Thus, prior knowledge of AGT levels in individual tumors could greatly facilitate the process of optimizing the efficacy of chemotherapy. In addition, assessing AGT levels in tumors *in vivo* would permit selection of patients most likely to benefit from such chemotherapy and would enable clinicians to monitor the effect of known and potential chemotherapeutic agents.

Related Prior Art

In vitro techniques to quantify AGT levels in tumor tissue have been described, but none of these are suitable for use in mammals *in vivo*. For example, Lee *et al.* (11) teaches immunohistochemical detection of AGT in human primary tumor samples and compares the staining intensity in these samples with levels of AGT activity.

Li, U.S. Patent 5,879,899, teaches methods of assaying DNA-repairing enzymes and their alkylated derivatives. The method employs a protease which can distinguish between the alkylated and non-alkylated forms of O^6-methylguanine-DNA-methyl-transferase (MGMT) in a blood or a tumor sample. Yarosh, U.S. Patent 5,407,804, teaches an assay for MGMT in tumor biopsy samples which uses monoclonal antibodies.

Ciocco *et al.* (43) discloses O^6-(*p*-hydroxy[^{3}H]methylbenzyl)guanine (in which the radiolabel is associated with a hydroxymethylbenzyl moiety) and its use as a reagent for detecting AGT in cultured cells using autoradiography.

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[1] The terms "BG" and "O^6-benzylguanine" are used interchangeably in this disclosure.

Derivatives of O^6-benzylguanine which inactivate AGT *in vitro* also have been described. Moschel *et al.* (16) discloses substituted (non-radiolabeled) benzylguanine molecules, particularly O^6-(*p*-fluorobenzyl)guanine, which inactivate AGT *in vitro* in human tumor cell extracts and intact tumor cells. Moschel *et al.* suggests administering the substituted benzylguanine molecules to enhance the cytotoxic effects of O^6-alkylating antitumor drugs. Moschel *et al.* does not disclose use of the substituted benzylguanine molecules to assess AGT levels *in vivo*.

Chae *et al.* (17) discloses O^6-benzylguanine derivatives, including O^6-(*p*-bromobenzyl)guanine, which inactivate AGT *in vitro*. Administration of the benzylguanine derivatives to enhance anti-tumor chemotherapy also is disclosed. Chae *et al.* suggests that the pharmacokinetics of AGT-inactivating agents could be altered, enabling the agents to be directed selectively to tumor cells. Again, the derivatives are not radiolabeled, and use of the derivatives to assess AGT levels *in vivo* is not taught or suggested.

Mountenou *et al.* (21) discloses O^6-4-[^{125}I]iodobenzylguanosine and its use as a substrate for AGT. Mountenou *et al.* teaches that the radiolabeled benzylguanosine derivative can be used to detect AGT in tumor cells to predict whether chloroethylating chemotherapeutic agents would be effective against a particular tumor. Mountenou *et al.* does not teach that the detection could be carried out *in vivo*.

Hotta *et al.* (10) teaches an assay of AGT activity in surgical samples of gliomas to predict the responsiveness of tumor cells to chloroethylnitrosoureas. The assay measures transfer of radioactivity from substrate DNA containing methyl-^{3}H-labeled O^6-methylguanine to an acid-insoluble protein fraction in crude tumor extracts. Hotta *et al.* does not teach or suggest assessing AGT levels *in vivo*.

Nesseler *et al.* (15) discloses synthesis of an ^{18}F-labeled guanine derivative with the ^{18}F attached at the N^9 position as a fluoroethyl group and its use for *in vivo* positron emission tomography imaging of MGMT. Binding of the ^{18}F-N^9-labeled derivative was evaluated *in vitro* in HeLa S3 cells; the derivative bound to MGMT with only a slightly decreased affinity compared to that of O^6-benzylguanine. However, despite the hope that, once dealkylated, the guanine residue would attach to the protein in a non-covalent fashion, the N^9-derivative was not demonstrated to transfer the radiolabel to the protein to make it

useful for AGT imaging.

Thus, there is a need in the art for methods which can be used to assess AGT levels in mammals *in vivo* in a noninvasive fashion.

SUMMARY OF THE INVENTION

It is an object of the present invention to provide reagents and methods for assessing AGT levels in mammals *in vivo*. These and other objects of the invention are provided by one or more of the embodiments described below.

One embodiment of the invention is a method of detecting O^6-alkylguanine-DNA alkyltransferase (AGT) molecules in a mammal. An AGT molecule in the mammal is contacted with an O^6-derivatized guanine molecule comprising at the exocyclic O^6 position a radiolabeled alkyl or benzyl moiety under conditions whereby the radiolabeled alkyl or benzyl moiety is transferred from the O^6-derivatized guanine molecule to the AGT molecule to form a radiolabeled AGT molecule. The radiolabeled AGT molecule is detected.

Another embodiment of the invention is a method of monitoring the effect of a reagent on the amount of AGT molecules in a tumor in a mammal. The reagent is administered to the mammal;. The amount of AGT molecules in the tumor is determined by (a) contacting AGT molecules in the tumor with O^6-derivatized guanine molecules comprising at the exocyclic O^6 position a radiolabeled alkyl or benzyl moiety, whereby radiolabeled alkyl or benzyl moieties are transferred from the O^6-derivatized guanine molecules to the AGT molecules to form radiolabeled AGT molecules, and (b) detecting the amount of radiolabeled AGT molecules in the tumor relative to a control in which no reagent is administered.

Still another embodiment of the invention is a composition useful for *in vivo* imaging of AGT molecules. The composition comprises radiolabeled O^6-benzylguanine molecule and a pharmaceutically acceptable carrier. The radiolabeled O^6-benzylguanine molecule is selected from the group consisting of 6-(4-[^{18}F]fluoro-benzyloxy)-9H-purin-2-ylamine, 6-(3-[^{131}I]iodo-benzyloxy)-9H-purin-2-ylamine, 6-(3-[^{125}I]iodo-benzyloxy)-9H-purin-2-ylamine, 6-(3-[^{123}I]iodo-benzyloxy)-9H-purin-2-ylamine, 6-(3-[^{124}I]iodo-

benzyloxy)-9H-purin-2-ylamine, 6-(4-[^{18}F]fluoro-3-nitrobenzyloxy)-9H-purin-2-ylamine, 6-(4-[^{131}I]iodo-benzyloxy)-9H-purin-2-ylamine, 6-(4-[^{125}I]iodo-benzyloxy)-9H-purin-2-ylamine, 6-(4-[^{123}I]iodo-benzyloxy)-9H-purin-2-ylamine, and 6-(4-[^{124}I]iodo-benzyloxy)-9H-purin-2-ylamine;

Thus, the invention provides reagents and methods for assessing AGT levels in mammals *in vivo*.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1. Depletion of cellular AGT activity by unlabeled 6-(4-fluoro-benzyloxy)-9*H*-purin-2-ylamine (*0*^{6}-4-fluorobenzylguanine (FBG) and 6-(iodo-benzyloxy)-9*H*-purin-2-ylamine (*0*^{6}-iodobenzylguanine (IBG). CHO cells transfected with pCMV-AGT were incubated with varying concentrations of IBG (■) or FBG (●) for 4 hours, and the AGT activity associated with the cells was determined. The results are expressed as the percentage of the AGT activity present in cell cultures that were not treated with FBG or IBG.

FIG. 2. Binding of [^{18}F]FBG to purified AGT as a function of unlabeled FBG concentration. [^{18}F]FBG was incubated for 30 minutes at 37 °C, in the presence or absence of increasing amounts of unlabeled FBG, with 10 μg of AGT (●), or to control for nonspecific binding, 10 μg of BSA (▲) in a Tris-buffer. The protein-associated activity was determined by TCA precipitation.

FIG. 3. Binding of [^{131}I]IBG to purified AGT as a function of unlabeled IBG concentration. The assay was performed as detailed under FIG. 2 by incubating [^{131}I]IBG with AGT (●) or BSA (▲).

FIG. 4. Scheme 1, preparation of ^{18}F-labeled compound 3 and coupling to the guanine skeleton.

FIG. 5. Scheme 2, preparation of compound 7 from compound 4 and commercially available 3-iodobenzyl alcohol in 60% isolated yield and converted to compound 8 by treatment with sodium hydride or potassium *tert*-butoxide, and SEM-Cl.

FIG. 6. Chart 1, preparation of compound 11 from the reported compound 10.

DETAILED DESCRIPTION OF THE INVENTION

It is a discovery of the present invention that AGT levels can be detected and measured *in vivo* using radiolabeled O^6-derivatized guanine molecules which bind selectively to AGT. Applications of this technique include the selection of patients most likely to benefit from alkylator therapy, monitoring the effect of these drugs on AGT levels during the therapeutic course, correlating therapeutic success with endogenous AGT, identifying test compounds as potential anti-cancer chemotherapeutic agents, and evaluating the clinical effectiveness of AGT-depleting agents.

Radiolabeled O^6-Derivatized Guanine Molecules

Isotopes such as ^{125}I, ^{123}I, ^{124}I, ^{131}I, and ^{18}F can be used to provide a radiolabeled O^6-derivatized guanine molecule. To be useful for imaging AGT levels, the radiolabel must reside within an alkyl or a benzyl moiety attached to the exocyclic O^6-position of the guanine, because this is the moiety that is transferred to the AGT molecule (16). This criterion is reinforced by the results of the study evaluating a ^{18}F-labeled guanine derivative with the ^{18}F attached at the N^9 position as a fluoroethyl group (15; *supra*); the guanine derivative labeled with ^{18}F at the N^9 position was ineffective for *in vivo* AGT imaging.

An O^6-derivatized guanine molecule useful in the present invention comprises at the exocyclic O^6 position a benzyl moiety or an alkyl moiety, such as an ethyl, n-propyl, or n-butyl moiety. Preferred moieties include fluoromethyl, fluoroethyl, fluoro-n-propyl, fluoro-n-butyl, *ortho*-fluoromethylbenzyl, *ortho*-fluoroethylbenzyl, *ortho*-fluoropropylbenzyl, *meta*-fluoromethylbenzyl, *meta*-fluoroethylbenzyl, *meta*-fluoropropylbenzyl, *para*-fluoromethylbenzyl, *para*-fluoroethylbenzyl, or *para*-fluoropropylbenzyl.

Preferably the O^6-derivatized guanine molecule is an O^6-benzylguanine molecule. A variety of substituents are tolerated in the benzene ring of O^6-benzylguanine (16). For example, FBG [6-(4-fluoro-benzyloxy)-9*H*-purin-2-ylamine; O^6-4-fluorobenzylguanine] is among the purine and pyrimidine derivatives that have been shown to be AGT depletors (16-20). The ability of FBG to deplete AGT in HT29 cell-free extracts and intact cells was shown to be similar to that of O^6-benzylguanine itself (16). Thus, a radiolabeled FBG, such

as ^{18}F-labeled FBG, can be a valuable tool for assessing the AGT levels; fluorine-18 has a 1.8 h half-life and is the most commonly used radionuclide for positron emission tomography.

Iodinated O^6-benzylguanine-derivatives also are useful in methods of the present invention. Iodine has a spectrum of radionuclides with different physical properties that are appropriate for external imaging. For example, ^{123}I ($t_{1/2}$= 13 h) is an excellent radionuclide for use in single photon emission tomography. Iodine-124 ($t_{1/2}$= 4.2 d) also has been used for PET. The preparation of radioiodinated guanosine derivatives has been described (21); however, the AGT binding properties of these derivatives have not been disclosed. While O^6-4-chlorobenzylguanine (CBG) and O^6-4-bromobenzylguanine (BBG) have been prepared and shown to be good analogues of BG (17), to our knowledge, the synthesis and biological activity of 6-(iodo-benzyloxy)-9*H*-purin-2-ylamine (O^6-iodobenzylguanines; IBG) have not been disclosed.

The present invention provides methods for the preparation of 3-IBG, and the exmplified labeled compounds 6-(4-[^{18}F]fluoro-benzyloxy)-9H-purin-2-ylamine([^{18}F]FBG) and 6-(3-[^{131}I]iodo-benzyloxy)-9H-purin-2-ylamine (3-[^{131}I]IBG), which are described in the specific examples below.

Administration of Compositions Comprising Radiolabeled O^6-Derivatized Guanine Molecules

Radiolabeled O^6-derivatized guanine molecules can be provided in a composition comprising a pharmaceutically acceptable carrier, such that the radiolabeled O^6-derivatized guanine molecules can be administered to a mammal. Pharmaceutically acceptable carriers are well known in the art and include physiologically compatible buffers such as Hanks' solution, Ringer's solution, dextrose, physiologically buffered saline, or water.

A composition of the invention can be administered by any suitable method which will provide the radiolabeled O^6-derivatized guanine molecules to a tumor. Tumors in which radiolabeled AGT molecules can be imaged include, but are not limited to, gliomas, glioblastomas, astrocytomas, medulloblastomas, Hodgkin's tumors, and tumors of the colon, breast, ovary, prostate, kidney, uterus, pancreas, lung, testis, and muscle.

Administration can be by any appropriate route to reach the desired tumor, including, but not limited to, oral, intravenous, intramuscular, intraarterial, intrathecal, intraventricular, transdermal, subcutaneous, epidural, intraperitoneal, intranasal, parenteral, topical, sublingual, or rectal means. Optionally, the composition can be injected directly into a tumor or into an organ in which a tumor is located, such as a breast, prostate, uterus, liver, kidney, pancreas, lung, or muscle.

Compositions of the invention can be administered to any mammal, including, but not limited to, dogs, cats, mice, rats, guinea pigs, horses, gorillas, chimpanzees, baboons, pigs, cows, monkeys, and humans. Under the physiological conditions in the mammalian body, the radiolabeled moiety attached through the exocyclic oxygen of an O^6-derivatized guanine molecule will be transferred to an AGT molecule; the result of this transfer is a radiolabeled AGT molecule which can be detected by a scintigraphic method.

The dose of the radiolabeled O^6-derivatized guanine molecule to be administered can be determined empirically, depending on the route of administration, the size of the mammal, and the type of tumor in which the level of AGT molecules is to be assessed. Generally, however, concentrations of radiolabeled O^6-derivatized guanine molecules in a composition of the invention will be in the range of no carrier added to 10 mg/kg, 10 to 25 mg/kg, 50 to 100 mg/kg, 75 to 200 mg/kg, 100 to 300 mg/kg, and 250 to 500 mg/kg. If desired, multiple administrations of a composition can be delivered.

Detecting Radiolabeled AGT Molecules

Any planar, dynamic, or tomographic scintigraphic method known in the art, such as planar imaging positron emission tomography (PET) or single photon emission tomography (SPECT), can be used to detect radiolabeled AGT molecules. PET is preferred as a detection method. PET is a functional imaging modality that can probe altered biochemical pathways and in some cases can be used to assess metabolic levels quantitatively (14). Thus, if desired, the number of radiolabeled AGT molecules can be quantitated. For example, the intensity of a signal generated by radiolabeled AGT molecules can be compared with the intensities of signals along a standard curve.

Identifying a Test Compound as a Potential Anti-Cancer Chemotherapeutic Agent

The method of the invention can be used to identify a test compound as a potential anti-cancer chemotherapeutic agent. Test compounds can be pharmacologic agents already known in the art or can be compounds previously unknown to have any pharmacological activity. The compounds can be naturally occurring or designed in the laboratory. They can be isolated from microorganisms, animals, or plants, and can be produced recombinantly, or synthesized by chemical methods known in the art. If desired, test compounds can be obtained using any of the numerous combinatorial library methods known in the art, including but not limited to, biological libraries, spatially addressable parallel solid phase or solution phase libraries, synthetic library methods requiring deconvolution, the "one-bead one-compound" library method, and synthetic library methods using affinity chromatography selection.

A test compound is administered to a mammal which bears a tumor. The tumor can be either experimentally induced or naturally occurring. A variety of tumor models suitable for use in this method are well known in the art, such as the murine colon 26-B carcinoma tumor model, the B16 mouse melanoma model, athymic mice bearing a D341MED human brain tumor xenograft, or nude mice injected with HT29 colon tumor cells, A172 glioblastoma cells, or human brain tumor cell lines such as SF767 and U251 MG. The test compound can be administered by any suitable means, including, but not limited to, oral, intravenous, intratumoral, intramuscular, intra-arterial, intramedullary, intrathecal, intraventricular, transdermal, subcutaneous, intraperitoneal, intranasal, parenteral, topical, sublingual, or rectal means.

The level of radiolabeled AGT in the tumor in response to the test compound is assessed as described above. If desired, radiolabeled AGT can be detected at multiple time points and/or after multiple administrations or concentrations of the test compound. A test compound which decreases the level of radiolabeled AGT molecules in the tumor relative to the absence of the test compound identifies the test compound as a potential anti-cancer chemotherapeutic agent. Preferably, the test compound decreases the level of radiolabeled AGT molecules in the tumor by at least 10, 25, 50, or 75%, preferably by at least 85, 90,

or 95%.

Monitoring the Effect of a Reagent on a Tumor

The effect on a tumor of a reagent, such as a test compound or a known chemotherapeutic agent, can be monitored by assessing the level of AGT in the tumor in response to the reagent. A test compound or a known chemotherapeutic agent is administered to a mammal which bears a naturally occurring or an experimentally induced tumor. Test compounds are those which are described above, including potential alkylating agent, and can be administered at doses ranging from 1 to 10 mg/kg, 5 to 50 mg/kg, 25 to 100 mg/kg, 75 to 200 mg/kg, 100 to 300 mg/kg, and 250 to 500 mg/kg. Known chemotherapeutic agents include, but are not limited to, alkylating agents such as chloroethylating nitrosoureas, such as 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU), 1-(4-amino-2-methyl-5-pyrimidinyl)methyl-3-(2-chloroethyl)-3-nitrosourea (ACNU), methyl-6-[3-(2-chloroethyl)-3-nitrosoureido]-6-deoxy-α-D-glucopyranoside(MCNU),CCNU,DTIC, temozolomide, and cyclophosphamide, as well as procarbazine, dacarbazine, and streptozotocin. Typical doses of such chemotherapeutic agents are well known in the art.

The level of radiolabeled AGT in the tumor in response to the test compound or known chemotherapeutic agent is assessed as described above. Optionally, multiple time points can be assayed; if desired, assays can be carried out after administering multiple concentrations or multiple doses of the same concentration of the test compound or chemotherapeutic agent.

The level of radiolabeled AGT molecules in the tumor relative to the absence of the reagent reflects the effect of the reagent on the tumor. Thus, a decrease in the level of radiolabeled AGT molecules of at least 10, 25, 50, 75, 85, 90, or 95% indicates a therapeutic effect of the reagent on the tumor.

All patents referenced in this disclosure are expressly incorporated by reference herein. The following examples are provided by way of illustration and are not intended to limit the scope of the invention, which is described by the appended claims.

EXAMPLE 1

Materials and Methods

All chemicals were purchased from Aldrich Chemical Company except as noted. FBG (unlabeled) was obtained as a gift from Dr. Robert Moschel of National Cancer Institute-Frederick Cancer Research and Development Center, Frederick, MD. Sodium [^{131}I]iodide in 0.1 N NaOH was supplied by DuPont-New England Nuclear (North Billerica, MA). [^{18}F]Fluoride activity was produced on the Duke University Medical Center CS-30 cyclotron via the $^{18}O(p,n)^{18}F$ reaction by irradiating [^{18}O]H_2O in a small volume (300 µl) silver target. The activity was delivered to a solution of Kryptofix (10 mg in 1 ml CH_3CN) and potassium carbonate (1 mg in 5 µl water) in a glass tube and then evaporated with argon in an oil bath at 80 °C. The dried activity was resolubilized in 50-100 µl of dry DMSO.

Melting points were determined on a Hake Buchler apparatus and were uncorrected. High pressure liquid chromatography (HPLC) was performed using one of three systems: 1) Two LKB Model 2150 pumps, an LKB Model 2152 control system, an LKB Model 2138 fixed-wavelength UV detector and a Beckman Model 170 radioisotope detector. Data were acquired using CIO-DAS08/JR internal and CIO-MINI137 external boards and Labtech DataLab/WIN software; 2) a Beckman System Gold HPLC equipped with a Model 126 programmable solvent module, a Model 168 diode array detector, a Model 170 radioisotope detector, and a Model 406 analog interface module; or 3) a Perkin-Elmer Series 4 Liquid Chromatograph connected to a Perkin-Elmer LC-95 UV/visible spectrophotometer detector and a Perkin-Elmer LCI-100 Laboratory Computing Integrator. Methods were programmed using a Perkin-Elmer 6312 display terminal.

For reversed-phase chromatography, a Waters µ Bondapak C18 (10 µm, 3.9 x 300 mm) column or a 3.9 x 300 mm Bondclone 10 (10 µ) C-18 column (Phenomenex, Torrance, CA) was used. Normal-phase HPLC was performed using a 4.6 x 250 mm Partisil (10 µ) (Alltech, Deerfield, IL).

Analytical TLC was performed on aluminum-backed sheets (Silica gel 60 F_{254}), and normal-phase column chromatography was performed using Silica gel 60, both obtained from EM Science (Gibbstown, NJ). Column chromatographic fractions were collected

using a Gilson model 203 micro fraction collector (Middleton, WI) or an ISCO Foxy 200 fraction collector (Lincoln, NE). Products identified by TLC. In some cases, an ISCO UA-6 UV-VIS detector was placed between the column outlet and the fraction collector to identify fractions.

Preparative thick layer chromatography was performed using 20 x 20 cm, 1000 μ plates (Whatman, Clifton, NJ). Before applying the sample, the plates were run in ethyl acetate to clean the plates of any absorbed impurities. Radio-TLC was initially analyzed using a System 200 Imaging Scanner (BioScan, Washington, D.C.) and then cut into strips and counted using an automatic gamma counter (LKB 1282, Wallac, Finland). NMR spectra (^{1}H-300 MHZ and ^{13}C-75 MHZ) were obtained on a Varian Mercury 300 spectrometer. Mass spectra were obtained on a Hewlett-Packard GC/MS/DS Model HP-5988A instrument, or on JEOL SX-102 high resolution mass spectrometer. Elemental analyses were provided by Galbraith Laboratories (Knoxville, TN).

EXAMPLE 2

Preparation of 6-(4-[^{18}F]Fluoro-benzyloxy)-9H-purin-2-ylamine ([^{18}F]FBG)

Para-[^{18}F]fluorobenzaldehyde was prepared following previously described procedures (26). Briefly, 50-100 mCi of [^{18}F]fluoride were resolubilized in DMSO (50-100 μl), added to 1-2 mg of 4-formyl-(*N,N,N*-trimethyl)anilinium trifluoromethane sulfonate in a 5-ml Reacti® vial. The mixture was heated in an oil bath at 150 °C for 10 minutes. The cooled reaction mixture was diluted with water and passed through an activated C18 solid-phase cartridge (Waters). The cartridge was further washed with water (5-10 ml), and 4-[^{18}F]fluorobenzaldehyde was finally eluted with methylene chloride (1-2 ml). The methylene chloride solution was dried with sodium sulfate and passed through a silica cartridge to remove any polar byproducts. Methylene chloride was removed using a rotary evaporator until a volume of about 0.1 ml was reached. Residual solution was transferred to a 1-dram vial. Use of argon to evaporate methylene chloride results in substantial loss of activity. Even with a rotary evaporator, it is important that the evaporation is stopped before the mixture dries.

DIBAL (0.1 ml, 1M solution in methylene chloride) was added to the above activity

and the capped vial was left at room temperature for 10 minutes. After evaporating the methylene chloride, 1 ml of water was added carefully to the residual activity followed by 50-100 µl concentrated HCl. The resultant 4-[^{18}F]fluorobenzyl alcohol was extracted from this solution with 3 x 1 ml of ethyl acetate. The combined ethyl acetate fractions were dried with sodium sulfate and concentrated on the rotary evaporator.

The above solution was transferred to a 1-ml Reacti® vial and evaporated to dryness. Potassium *tert*-butoxide (50 µl; 1M in THF) was added to this dried activity, and THF was evaporated under an argon flow. The residue was resolubilized in dry DMSO (Pierce, Rockford, IL; 50 µl) and the capped vial was heated at 100 °C for 5 minutes. The quaternary salt **4** (29) (1-2 mg) was added to the above solution, and the mixture was heated for an additional 10 minutes at 100 °C. [^{18}F]FBG ([^{18}F]**6**; t_R = 18-20 minutes) was isolated from this reaction mixture by reversed-phase HPLC (system 1) using the Bondclone column eluted with 0.1% TFA in 15:85 acetonitrile:water at a flow rate of 1 ml/min. The HPLC fractions containing [^{18}F]**6** were evaporated with argon to remove most of the acetonitrile, diluted with water, and passed through a solid-phase cartridge (tC18 ENV Sep Pak; Waters) activated with ethanol and water. The cartridge was washed with 5 ml of 0.9% saline, and the activity was eluted with 0.25 ml portions of ethanol. Most of the activity elutes in fractions 3-5. These pooled ethanol fractions were concentrated. For *in vitro* assays, the activity was reconstituted in Tris-buffer.

EXAMPLE 3

Preparation of 6-(3-Iodo-benzyloxy)-9H-purin-2-ylamine (3-IBG)

Sodium hydride (60% in mineral oil; 80 mg, 2 mmol) was added to a solution of 3-iodobenzyl alcohol (498 mg, 2.13 mmol) in dry DMSO (0.5 ml), and the resultant yellow solution was stirred at room temperature for 1 hour. The quaternary salt **4** (229 mg, 1 mmol) was added to the mixture, and the stirring was continued for another 2 hours. Acetic acid (170 µl) was added to the clear, homogeneous reaction mixture, which was then added with vigorous stirring to about 30 ml of ether. The resultant precipitate was filtered, washed with ether and then with water, and dried over P_2O_5 under vacuum to give 218 mg (60%) of **7**: mp 118-120 °C; HPLC (System 3): Bondapak column was eluted with 0.1%

TFA in 30:70 acetonitrile:water at 1 ml/min; t_R = 6.0 minutes as compared to 8-9 minutes for 3-iodobenzyl alcohol; ^{1}H-NMR (DMSO-d_6) 5.46 (s, 2H, benzylic), 6.28 (br s, 2H, NH_2), 7.21 (t, 1H, *m*-ArH; J_{H-H} = 8.5), 7.52 (d, 1H, *o*-ArH; J_{H-H} = 8.5), 7.72 (d, 1H, *p*-ArH; J_{H-H} = 8.5), 7.84 (s, 1H, *o'*-ArH), 7.87 (s, 1H, H-8), 12.45 (br s, 1H, NH); ^{13}C-NMR (CD_3OD) 67.77 (benzylic), 97.40 (ArC-I), 128.37 (guanine C-5 and *o*-ArCH), 131.19 (*o'*-ArCH and *p*-ArCH), 137.99 (*m*-ArCH and *ipso*-ArC), 138.07 (guanine CH-8 and C-6), 140.34 (guanine C-2 and C-4); MS (FAB$^+$) *m/z*: 368 (MH^+). HRMS calculated for $C_{12}H_{11}IN_5O$ (MH+) *m/z*: 368.0008. Found: 368.0013 ± 0.0009. Analyzed $C_{12}H_{10}IN_5O$ C, H, N: calculated, C, 39.26; H, 2.75; N, 19.08. Found, C, 39.03; H, 2.87, N, 18.17.

EXAMPLE 4

Preparation of 6-(3-Iodo-benzyloxy)-9-(2-trimethylsilanyl-ethoxymethyl)-9H-purin-2-ylamine (SEM-3-IBG)

THF was evaporated from a 1M solution of potassium *tert*-butoxide in THF (0.83 ml, 830 mmol). An equivalent amount of sodium hydride was used with similar results. To the residue was added 7 (300 mg, 0.82 mmol) followed by DMF (silylation grade; Pierce, 4.2 ml). The mixture was stirred at room temperature under argon for 1 hour, cooled to 0 °C, and SEM-chloride (138 mg, 0.83 mmol) was added drop-wise. The reaction was allowed to proceed overnight and was worked up by partitioning between ethyl acetate and water. Silica gel chromatography using 40:60 ethyl acetate:hexane afforded 105 mg (26%) of 8 as an oil/low melting solid: ^{1}H-NMR ($CDCl_3$) -0.02 (s, 9H, -$SiMe_3$), 0.93 (m, 2H, -Si-CH_2-), 3.58 (m, 2H, Si-CH_2-CH_2-O-), 4.83 (br s, 2H, NH_2), 5.44 (s, 2H, benzylic), 5.50 (s, 2H, -N-CH_2-O-), 7.09 (t, 1H, *m*-ArH; J_{H-H} = 7.7), 7.48 (d, 1H, *o*-ArH; J_{H-H} = 7.5), 7.65 (d, 1H, *p*-ArH; J_{H-H} = 7.8), 7.74 (s, 1H, H-8), 7.86 (s, 1H, *o'*-ArH), ^{13}C-NMR ($CDCl_3$); -2.05 (-$SiMe_3$), 17.06 (-CH_2-$SiMe_3$), 66.17 (-Si-CH_2-CH_2-O-), 66.28 (benzylic), 71.14 (-N-CH_2-O-), 93.41 (C-I), 112.48 (guanine C-5), 126.57 (*o*-ArCH), 129.29 (*m*-ArCH), 136.20 (*o'*- and *p*-ArCH), 137.86 (ArCH-*ipso*), 138.72 (guanine CH-8), 153.66 (guanine C-6), 158.52 (guanine C-2), 159.87 (guanine C-4); HRMS (FAB$^+$) calculated for $C_{18}H_{25}IN_5O_2Si$ (MH^+) *m/z*: 498.0822. Found: 498.0820 ± 0.0018. Analyzed $C_{18}H_{24}IN_5O_2Si$ C, H, N: calculated, C, 43.46; H, 4.86; N, 14.08. Found, C, 43.56; H, 4.69,

N, 13.84.

EXAMPLE 5

Preparation of 9-(2-Trimethylsilanyl-ethoxymethyl)-6-(3-trimethylstannanyl-benzyloxy)-9H-purin-2-ylamine

A mixture of 8 (50 mg, 0.1 mmol), hexamethylditin (210 mg, 0.64 mmol) and dichlorobis(triphenylphospine)palladium(II) (35 mg, 0.05 mmol) in 3 ml of dioxane was heated at reflux under argon for 1 hour. Dioxane was removed from the dark reaction mixture, and the residue was resuspended in ethyl acetate. The suspension was filtered through a bed of Celite, and the bed was washed thoroughly with ethyl acetate. The filtrate was concentrated to obtain an oil. This oil was applied to a bed of silica gel, and very non-polar impurities were removed by eluting with hexane. The required product and other byproducts were isolated from the silica gel bed by eluting with ethyl acetate. Ethyl acetate was removed from this solution. The residual oil was subjected to preparative TLC using 40:60 ethyl acetate:hexane to obtain 39 mg (73%) of 9 as a white solid: mp 135-136 °C, ^{1}H-NMR ($CDCl_3$) -0.03 (s, 9H, -$SiMe_3$), 0.29 (s, 9H, -$SnMe_3$), 0.92 (m, 2H, -$\underline{CH_2}$-$SiMe_3$), 3.57 (m, 2H, -Si-CH_2-$\underline{CH_2}$-O-), 4.87 (br s, NH_2), 5.43 (s, 2H, benzylic), 5.55 (s, 2H, -N-$\underline{CH_2}$-O-), 7.31-7.61 (m, 4H, ArH), 7.72 (s, 1H, H-8), ^{13}C-NMR ($CDCl_3$) -9.37 (-$SiMe_3$), -1.32 (-$SnMe_3$) 17.80 (-$\underline{CH_2}$-$SiMe_3$), 66.87 (-Si-CH_2-$\underline{CH_2}$-O-), 68.47 (benzylic), 71.84 (-N-$\underline{CH_2}$-O-), 127.90 (guanine C-5), 128.28 (*o*-ArCH), 128.56 (*m*-ArCH), 129.24 (*o'*- and *p*-ArCH), 135.47 (ArCH-*ipso*), 135.60 (C-Sn), 135.94 (guanine CH-8), 139.27 (guanine C-6), 154.28 (guanine C-2), 159.31 (guanine C-4); MS (FAB$^+$) calculated for $C_{21}H_{33}IN_5O_2Si^{120}Sn$ (M^+) *m/z*: 535.1429. Found: 535.1429 ± 0.0001. Analyzed $C_{21}H_{33}IN_5O_2SiSn$ C, H, N: calculated, C, 47.21; H, 6.23; N, 13.11. Found, C, 47.35; H, 6.09, N, 12.89.

EXAMPLE 6

Preparation of 6-(3-[^{131}I]Iodo-benzyloxy)-9H-purin-2-ylamine(3-[^{131}I]IBG)

A. Two-step approach

To a ½-dram vial containing 0.2 mg of 9 was added 1-2 µl of ^{131}I in 0.1N NaOH (about 1 mCi), followed by 10 µl of a 3:1 (v/v) solution of acetic acid:30% H_2O_2. The

mixture was sonicated for about 30 seconds, injected onto a normal-phase HPLC column, and eluted with 0.2% acetic acid in 50:50 ethyl acetate:hexane at a flow rate of 1 ml/minute. The required product [^{131}I]8 (t_R = ~13 minutes) was isolated in more than 90% radiochemical yield.

Solvents from HPLC fractions containing [^{131}I]8 were evaporated to a small volume, transferred to a ½-dram vial. The solvents were again evaporated to dryness. The residual radioactivity was treated with trifluoroacetic acid (50 µl) for 5 minutes at room temperature. Most of the trifluoroacetic acid was evaporated with an argon flow and triturated with 50 µl of ethyl acetate twice to insure its complete removal. Methanolic ammonia (50 µl) was added to the vial. The vial was vortexed, and methanol and ammonia were evaporated off under a flow of argon. The radioactivity was reconstituted in methanol and injected onto a normal-phase HPLC column eluted with 0.1% acetic acid in ethyl acetate at a flow rate of 1 ml/min. The desired [^{131}I]7 (t_R = ~13 minutes) was isolated in 55-60% radiochemical yield. Under these HPLC conditions, neither 3-iodobenzyl alcohol nor **8** were retained in the column. To ensure the authenticity of the final product, it was subjected to TLC in 5% (v/v) methanol in ethyl acetate along with the unlabeled standard. The retention factor for **7** was 0.3, while those for 3-iodobenzyl alcohol and **8** were 0.8, 0.9, respectively.

The deprotection of [^{131}I]8 to [^{131}I]7 also was accomplished with tetrabutylammonium fluoride (TBAF) in THF. For this, TBAF (50 µl, 1 M in THF) was added to a vial containing the residue of [^{131}I]8, and the capped vial was heated at 65-70 °C for 25 minutes. THF was evaporated. The activity was reconstituted in a mixture of 25 µl methanol and 5 µl acetic acid and injected on to a normal-phase HPLC as described above. The radiochemical yield was 85-90%.

B. Single-step approach

After radioiodination of **8**, solvents were removed by co-evaporating twice with a few microliters of benzene. The residual activity was heated with 50 µl of TBAF in THF for 25 minutes at 65-70 °C . After evaporating THF, the activity was processed as above, and [^{131}I]7 was isolated by normal-phase HPLC in 70% radiochemical yield.

EXAMPLE 7

Cell Culture and Transfection

CHO-K1 cells were maintained in α MEM (Gibco, Grand Island, NY) containing 10% fetal bovine serum. CHO cells were transfected with a plasmid expressing human AGT (46) using FuGENE (Roche Molecular Biochemicals) according to the manufacturer's protocol for transfection of adherent cells. After 48 hours, geneticin (Gibco) was added at a concentration of 500 μg/ml. Clones were isolated from individual cell foci.

EXAMPLE 8

AGT Activity Assays

Inactivation of cellular AGT was measured by adding varying concentrations of FBG or IBG to cell cultures that had reached 80-90% confluence. After 4 hours of drug treatment, cell extracts were prepared. AGT activity was measured by assaying the loss of [^{3}H]-O^6-methylguanine from a [^{3}H] methylated calf thymus DNA substrate as described (6). The results are expressed as the percentage of the AGT activity present in cell cultures that were not treated with FBG or IBG. For each concentration, the assay was performed in duplicate.

EXAMPLE 9

In Vitro *Binding of FBG or IBG by AGT*

Purified human AGT with a $(His)_6$ tag (47) was generously provided by Dr. Anthony E. Pegg of Hershey Medical Center, Pennsylvania State University. About 50,000 counts of [^{18}F]FBG or [^{131}I]IBG were added in the presence or absence of increasing amounts of cold FBG or IBG, respectively, to 10 μg AGT or to a control for nonspecific binding, BSA. The incubation carried out in 0.1 ml 50 mM Tris-Cl, pH 7.5, 5 mM DTT, 0.1 mM EDTA in the presence of 10 μg calf thymus DNA. After incubation for 30 minutes at 37 °C, the protein was precipitated by the addition of 200 μg carrier BSA and 1 ml cold 12% TCA. The precipitated proteins were collected on GF-C (Whatman) filters, which were washed extensively with 5% TCA. The results are expressed as the percentage of

input activity retained on the filter. For each concentration, the assay was performed in triplicate. The assay was performed twice for both [^{18}F]FBG and [^{131}I]IBG.

EXAMPLE 10

Depletion of AGT Activity by FBG and IBG

Because IBG is a new compound, its ability to deplete AGT was determined using CHO cells transfected with pCMV-AGT to express AGT. In parallel, the ability of FBG to deplete AGT under the same conditions also was measured. As shown in FIG. 1, IBG depleted AGT from these cells to a degree higher than that depleted by FBG. An IC_{50} of 50 nM was measured for FBG, similar to the value reported for FBG in HT29 human colon tumor cells (16). On the other hand, IBG had an IC_{50} of 15 nM, suggesting that it is more potent than FBG in depleting AGT. These results demonstrate that introduction of an iodine at the *meta*-position of the benzyl group in BG did not result in a loss of AGT-depleting ability.

EXAMPLE 11

Binding of radiolabeled FBG and IBG to purified AGT

To investigate whether AGT recognizes radiolabeled FBG and IBG as substrates, the efficacy of transfer of their radio labeled benzyl groups to AGT was determined using the purified protein. For [^{18}F]FBG, the percent of input activity that bound to AGT increased with increasing concentrations of unlabeled FBG reaching a maximum of about 90% at 0.5 μM of FBG (FIG. 2). Saturation was observed at concentrations higher than 2 μM. An IC_{50} of 5 μM was calculated. About 60% of radio labeled IBG bound to AGT over a wide range of concentrations (FIG. 3) with saturation noticed at concentrations higher than 3 μM. The IC_{50} for IBG was about 6 μM. These relatively high IC_{50} values further suggest that higher specific activity will not be a requirement for these radiopharmaceuticals. In both cases, the binding was specific as demonstrated by the fact that very little radioactivity was bound to the nonspecific protein BSA with maximum observed AGT to BSA binding ratios of 661 and 35 for [^{18}F]FBG and [^{131}I]FBG, respectively. These results are qualitatively similar to that obtained with O^6-(*p*-

hydroxy[^{3}H]methylbenzyl)guanine, the only other BG derivative labeled at the benzyl part of the molecule (1, 43). The preparation of ^{13}C- and ^{14}C-labeled O^6-benzylguanosines has been reported (44).

Although IBG depletes AGT from cells to a greater degree than FBG, [^{18}F]FBG had a higher binding to the purified protein than [^{131}I]IBG. This may be due to differential transmembrane transport of the two compounds as a result of differences in their lipophilicity. The lipophilicities of IBG and FBG were not determined *per se*. Intuitively, IBG is expected to be more lipophilic. Reversed-phase HPLC has been used to determine lipophilicities (45). In comparison to FBG, a higher percentage of acetonitrile was needed to elute IBG from a revered-phase column, suggesting that IBG is more lipophilic. However, it should be pointed out that the more non-polar 4-fluorobenzyl alcohol elutes earlier (t_R = 10-11 minutes) than FBG (t_R = 18-20 minutes) under identical reversed-phase HPLC conditions.

Discussion

It is highly desirable that radiochemical syntheses be performed with the least number of steps possible, particularly for short half-life radionuclides such as ^{18}F (110 minutes). A prerequisite for insertion of ^{18}F onto a benzene ring by nucleophilic substitution is that the ring contains a suitable leaving group, such as -NO_2 or a quaternary ammonium triflate, that is positioned *ortho* or *para* to a strongly electron withdrawing group such as NO_2 (22, 23). It may be possible to prepare an FBG precursor, such as the one with an -NO_2 or a quaternary ammonium group in the place of fluorine, from which [^{18}F]FBG may be produced in a single step. However, this chemistry will not be facile due to the lack of a suitably placed electron withdrawing group in the molecule. To circumvent this, it was necessary to first prepare ^{18}F-labeled 3 and couple it to the guanine skeleton (Scheme 1; FIG. 4). Although the chemistry of [^{18}F] 3 has been reported (24, 25), a slightly different set of conditions was used for its preparation. *Para*-[^{18}F]fluorobenzaldehyde, [^{18}F]2, was prepared as reported (26). Reduction of [^{18}F]2 was initially accomplished with $NaBH_4$ in ethanol at 80°C. However, this involved removal of the initial solvent methylene chloride from a solution of [^{18}F]2 and reconstitution of [^{18}F]2 in ethanol. In addition to the

extra time needed, this process resulted in a considerable loss of activity due to the very volatile nature of [^{18}F]2. It was found that a commercially available solution of DIBAL in methylene chloride was very effective in converting [^{18}F]2 to [^{18}F]3 at room temperature. It should be pointed out that the development of a technique to prepare [^{18}F]3 by on-column reduction of [^{18}F]2 using $NaBH_4$ has been reported very recently (27). Using our procedure, preparation of [^{18}F]3 from [^{18}F]fluoride could be accomplished in about an hour in an overall decay-corrected radiochemical yield of 50%.

Unlabeled FBG was originally prepared by the treatment of 2-amino-6-chloropurine (ACP) at 100-130 °C for over 24 hours with an excess of the sodium salt of 4-fluorobenzyl alcohol in 4-fluorobenzyl alcohol as solvent (16). These conditions are not adaptable to ^{18}F labeling. Even if one started with Curie-quantities of [^{18}F]3 and microgram amounts of ACP, the concentration of 3 would be substantially sub-stoichiometric. In addition, the long reaction time is not suitable with ^{18}F. We initially attempted this chemistry by conducting the reaction in a solvent such as THF or DME without success. A number of O^6-hetarylguanines have been prepared in excellent yields based on an earlier report (28) using 4 (29), instead of ACP, as the starting material (20). These reactions proceeded under mild conditions and involved easy work-up. The procedure essentially involves the treatment of 4 with the pre-formed sodium alcoholate (2 equivalents of sodium hydride and 5.6 equivalents of desired alcohol) in DMSO at room temperature for 1 hour. We have shown that treatment of the pre-formed sodium salt of 4-fluorobenzyl alcohol (using sodium hydride) with an equimolar quantity of 4 in DMSO at room temperature resulted in the formation of 6 in more than 80% yield (HPLC) in 30 minutes. It was also possible to convert ACP to 6 by doing the reaction in DMSO, albeit at a higher temperature. Treatment of ACP with 2 equivalents of the potassium salt of 4-fluorobenzyl alcohol (using potassium *tert*-butoxide) in DMSO at room temperature did not result in the formation of 6. However, heating the reaction mixture at 130-140 °C for 30 minutes resulted in about 40% conversion, determined by HPLC, of ACP to 6.

A higher reaction temperature was used to augment the rate of the radiochemical reaction of [^{18}F]3 with 4. Typically, [^{18}F]3 was heated with a base in DMSO for 5 minutes at 100 °C (to ensure deprotonation), and heated again for 10 minutes after the addition of

4. Sodium hydride was initially used as a base, but owing to cumbersome handling of sodium hydride, a readily available and easily measurable solution of potassium *tert*-butoxide in THF was subsequently used, giving equally good results. Although in some cases decay-corrected radiochemical yields of about 70% have been obtained for this coupling reaction, the yield generally was around 40%. In a few reactions, ACP was substituted for 4; however, the decay-corrected radiochemical yield was only about 25%. Little or no radioactivity corresponding to [^{18}F]3 eluted in reversed-phase HPLC. The remainder of the radioactivity was retained in the column, suggesting the possibility of highly non-polar byproduct(s). About 7-9 mCi of [^{18}F]6 was obtained starting from 100 mCi of resolubilized [^{18}F]fluoride in about 2 hours. Although the specific activity of the final product has not been systematically determined, no co-eluting carrier peak was generally observed on HPLC. Nonetheless, unlike receptor-based radiopharmaceuticals, very high specific activity is not necessary for O^6-alkyl guanine derivatives and may even be counterproductive because transfer of alkyl groups by AGT follows second order kinetics (30, 31).

One of the most commonly used techniques for the preparation of radioiodinated compounds is the radioiododestannylation of the corresponding tin precursor. Initially, we envisaged the preparation of **11** (Chart I, FIG. 6) from the reported compound **10** (17). It may be possible to prepare **12** or its radioiodinated analogue by the halodestannylation of **11**; however, attempts to convert **10** to **11** by treatment with hexamethylditin and bis-triphenylphosphine palladium dichloride in dioxane were unsuccessful. This was probably due to the insolubility of **10** in dioxane.

Guanine chemistry is often challenging due to the amphoteric nature of guanine derivatives (32). Chemical manipulations can be made more facile if the starting compound can be converted to a non-polar derivative by the introduction of a protecting group. For example, this strategy has simplified the preparation of polar guanidines (33). Guanine derivatives modified with different moieties at the N^9-position have been reported (34-36). These derivatives have sufficient non-polar nature to permit purification by silica gel chromatography. Although some of these N^9-substituents (34, 36) may be potentially used as protecting groups, the reported work was not done with that objective. Protecting

group methodology has been applied to adenine chemistry (37); however, to our knowledge, such a strategy has not been reported for guanine derivatives themselves. We prepared several ACP surrogates with protecting groups, such as trimethylsilylethyl, trimethylsilylethyoxymethyl (SEM), *tert*-butyloxymethyl, and pivaloyloxymethyl, at the N^9-position, and converted some of these to their O^6-(substituted)benzyl derivatives. For the present work, we used the SEM protecting group. Recently, SEM and *p*-methoxyphenylmethyl (MPM) protecting groups were used to facilitate the coupling of 6-halopurine to glycosylamines (38).

Although it should have been possible to convert N^9-SEM-ACP to 8 (*vide supra*), we used an alternative approach following the procedure of McElhinney *et al.* (20) (Scheme 2). Compound 7 was prepared from 4 and commercially available 3-iodobenzyl alcohol in 60% isolated yield and converted to 8 by treatment with sodium hydride or potassium *tert*-butoxide, and SEM-Cl. The unoptimized isolated yield of 8 from this reaction was only 26%. In contrast, yields of 50-85% have been reported for modification of amines with a SEM Group (39-41). The N^7-regioisomer is a byproduct in the *N*-alkylation of guanines; however, the N^9/N^7 ratio generally is very high (35). We did not attempt to isolate the N^7-regioisomer of 8 if any was formed. Compound 8 was smoothly converted to 9 using the palladium-catalyzed stannylation.

Radioiodination of 9 to [^{131}I]8 was performed easily by sonication for 30 seconds with ^{131}I and a mixture of acetic acid and hydrogen peroxide. The radiochemical yield was more than 90%. Tetrabutylammonium fluoride (TBAF) and TFA are among reagents that have been used for the removal of the SEM group. We initially used TFA for converting [^{131}I]8 to [^{131}I]7 based on preliminary results for the deprotection of N^9-(trimethylsilylethyl) ACP (TMSE-ACP) to ACP. TMSE-ACP was treated with TBAF in DMF or DMSO at room temperature and at 60°C. TMSE-ACP remained intact at room temperature for at least one hour. Analysis of the reaction mixtures left at room temperature for 20 hours and those heated at 60°C for 5 minutes by HPLC showed several by-product peaks. On the other hand, a single product peak corresponding to ACP was observed when TMSE-ACP was treated with TFA for 5 minutes at room temperature. Treatment of [^{131}I]8 with TFA at room temperature for 5 minutes gave 55-60% radiochemical yield of [^{131}I]7; 15-20% of

unreacted [^{131}I]8 was recovered. Conducting the reaction for a longer time did not improve the radiochemical yield.

A combination of radio-TLC, and normal-phase and reversed-phase HPLC was used to verify that the final product was indeed IBG and not the 3-iodobenzyl alcohol. Very little if any of 3-iodobenzyl alcohol was detected in the reaction mixture. In addition, the final product bound to AGT specifically (*vide infra*), which further supports its authenticity. It was surprising that TFA treatment did not cleave the ethereal linkage between guanine and the benzyl moieties because the instability of BG to acidic conditions has been reported (42). This may be due to the lack of water in the reaction mixture or a rate factor may be involved. It should be pointed out that both IBG and FBG were stable during reversed-phase chromatography involving 0.1% TFA in water (pH 2) as a co-solvent.

No deprotection was observed when [^{131}I]8 was treated with a 1 M solution of TBAF in THF at room temperature up to 30 minutes, and [^{131}I]8 was recovered quantitatively. Increasing the reaction temperature to 70 °C resulted in the formation of [^{131}I]7 in a time-dependent fashion. A maximum radiochemical yield of 85% was obtained after about 25 minutes, at which time essentially only one radioactive peak corresponding to IBG was seen on HPLC. This is contrary to the results described above for the deprotection of TMSE-ACP with TBAF at macromolar levels. Possible reasons for this are the presence of a better leaving group at the O^6-position in TMSE-ACP and the difference in reaction rate at macromolar and tracer levels.

The above procedures involve two normal-phase HPLC separations. Although it was possible to perform the two steps in the same pot consecutively, there was an unlabeled peak co-eluting with the desired radioactive peak when the final mixture was subjected to normal-phase HPLC. This was presumably **13** (Chart 1), because the same peak was seen when **9** was treated with TBAF and injected onto the HPLC. Use of reversed-phase HPLC may be necessary to separate **7** and **13**. However, isolating [^{131}I]7 from reversed-phase HPLC fractions involves a solid-phase extraction and removal of a solvent such as ethanol or methanol. Because it is much easier to evaporate ethyl acetate, the two normal-phase HPLC procedure is preferred. Preparation of **13** in sufficient quantities should permit direct

conversion of 13 to [^{131}I]7 in a single step.

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CLAIMS

1. A method of detecting O^6-alkylguanine-DNA alkyltransferase (AGT) molecules in a mammal, comprising the steps of:

contacting an AGT molecule in the mammal with an O^6-derivatized guanine molecule comprising at the exocyclic O^6 position a radiolabeled alkyl or benzyl moiety under conditions whereby the radiolabeled alkyl or benzyl moiety is transferred from the O^6-derivatized guanine molecule to the AGT molecule to form a radiolabeled AGT molecule; and

detecting the radiolabeled AGT molecule.

2. The method of claim 1 wherein the amount of radiolabeled AGT molecules is determined.

3. The method of claim 1 wherein the AGT molecule is present in a tumor.

4. The method of claim 3 wherein the tumor is selected from the group consisting of a glioma, a glioblastoma, a medulloblastoma, an astrocytoma, a Hodgkin's tumor, a colon tumor, a breast tumor, an ovarian tumor, a prostate tumor, a kidney tumor, a melanoma, a liver tumor, a uterine tumor, a pancreatic tumor, a lung tumor, a testicular tumor, and a muscle tumor.

5. The method of claim 1 wherein the radiolabeled AGT molecule is detected by a scintigraphic method.

6. The method of claim 5 wherein the scintigraphic method is positron emission tomography.

7. The method of claim 1 wherein the radiolabeled alkyl moiety is selected from the group consisting of fluoromethyl, fluoroethyl, fluoro-n-propyl, fluoro-n-butyl, *ortho*-fluoromethylbenzyl, *ortho*-fluoroethylbenzyl, *ortho*-fluoropropylbenzyl, *meta*-fluoromethylbenzyl, *meta*-fluoroethylbenzyl, *meta*-fluoropropylbenzyl, *para*-fluoromethylbenzyl, *para*-fluoroethylbenzyl, and *para*-fluoropropylbenzyl.

8. The method of claim 1 wherein the radiolabeled benzyl moiety is selected from the group consisting of *ortho*-fluoromethylbenzyl, *ortho*-fluoroethylbenzyl, *ortho*-fluoropropylbenzyl, *meta*-fluoromethylbenzyl, *meta*-fluoroethylbenzyl, *meta*-fluoropropylbenzyl, *para*-fluoromethylbenzyl, *para*-fluoroethylbenzyl, and *para*-

fluoropropylbenzyl.

9. The method of claim 1 wherein the O^6-derivatized guanine molecule is an O^6-benzylguanine molecule.

10. The method of claim 9 wherein the O^6-benzylguanine molecule is selected from the group consisting of 6-(4-[^{18}F]fluoro-benzyloxy)-9H-purin-2-ylamine, 6-(3-[^{131}I]iodo-benzyloxy)-9H-purin-2-ylamine,6-(3-[^{125}I]iodo-benzyloxy)-9H-purin-2-ylamine, 6-(3-[^{123}I]iodo-benzyloxy)-9H-purin-2-ylamine, 6-(3-[^{124}I]iodo-benzyloxy)-9H-purin-2-ylamine, 6-(4-[^{18}F]fluoro-3-nitrobenzyloxy)-9H-purin-2-ylamine, 6-(4-[^{131}I]iodo-benzyloxy)-9H-purin-2-ylamine, 6-(4-[^{125}I]iodo-benzyloxy)-9H-purin-2-ylamine, 6-(4-[^{123}I]iodo-benzyloxy)-9H-purin-2-ylamine, and 6-(4-[^{124}I]iodo-benzyloxy)-9H-purin-2-ylamine.

11. The method of claim 1 wherein the mammal is a human.

12. The method of claim 1 wherein the radiolabeled alkyl or benzyl moiety comprises a radiolabel selected from the group consisting of ^{125}I, ^{123}I, ^{124}I, ^{131}I, and ^{18}F.

13. A method of monitoring the effect of a reagent on the amount of AGT molecules in a tumor in a mammal, comprising the steps of:

administering the reagent to the mammal; and

determining the amount of AGT molecules in the tumor by

(a) contacting AGT molecules in the tumor with O^6-derivatized guanine molecules comprising at the exocyclic O^6 position a radiolabeled alkyl or benzyl moiety, whereby radiolabeled alkyl or benzyl moieties are transferred from the O^6-derivatized guanine molecules to the AGT molecules to form radiolabeled AGT molecules; and

(b) detecting the amount of radiolabeled AGT molecules in the tumor relative to a control in which no reagent is administered.

14. The method of claim 13 wherein the reagent is an alkylating agent.

15. The method of claim 13 wherein the reagent is a test compound, wherein a decrease in the amount of radiolabeled AGT molecules in the tumor after administration of the test compound identifies the test compound as a potential anti-cancer chemotherapeutic agent.

16. The method of claim 13 wherein the reagent is a known chemotherapeutic agent.

17. The method of claim 13 wherein the tumor is selected from the group consisting of a glioma, a glioblastoma, a medulloblastoma, an astrocytoma, a Hodgkin's tumor, a colon tumor, a breast tumor, an ovarian tumor, a prostate tumor, a kidney tumor, a melanoma, a liver tumor, a uterine tumor, a pancreatic tumor, a lung tumor, a testicular tumor, and a muscle tumor.

18. The method of claim 13 wherein the amount of radiolabeled AGT molecules is detected by a scintigraphic method.

19. The method of claim 18 wherein the scintigraphic method is positron emission tomography.

20. The method of claim 13 wherein the radiolabeled alkyl moiety is selected from the group consisting of fluoromethyl, fluoroethyl, fluoro-n-propyl, fluoro-n-butyl, *ortho*-fluoromethylbenzyl, *ortho*-fluoroethylbenzyl, *ortho*-fluoropropylbenzyl, *meta*-fluoromethylbenzyl, *meta*-fluoroethylbenzyl, *meta*-fluoropropylbenzyl, *para*-fluoromethylbenzyl, *para*-fluoroethylbenzyl, and *para*-fluoropropylbenzyl.

21. The method of claim 13 wherein the radiolabeled benzyl moiety is selected from the group consisting of *ortho*-fluoromethylbenzyl, *ortho*-fluoroethylbenzyl, *ortho*-fluoropropylbenzyl, *meta*-fluoromethylbenzyl, *meta*-fluoroethylbenzyl, *meta*-fluoropropylbenzyl, *para*-fluoromethylbenzyl, *para*-fluoroethylbenzyl, and *para*-fluoropropylbenzyl.

22. The method of claim 13 wherein the O^6-derivatized guanine molecule is an O^6-benzylguanine molecule.

23. The method of claim 22 wherein the O^6-benzylguanine molecules are selected from the group consisting of 6-(4-[^{18}F]fluoro-benzyloxy)-9H-purin-2-ylamine, 6-(3-[^{131}I]iodo-benzyloxy)-9H-purin-2-ylamine, 6-(3-[^{125}I]iodo-benzyloxy)-9H-purin-2-ylamine, 6-(3-[^{123}I]iodo-benzyloxy)-9H-purin-2-ylamine, 6-(3-[^{124}I]iodo-benzyloxy)-9H-purin-2-ylamine, 6-(4-[^{18}F]fluoro-3-nitrobenzyloxy)-9H-purin-2-ylamine, 6-(4-[^{131}I]iodo-benzyloxy)-9H-purin-2-ylamine, 6-(4-[^{125}I]iodo-benzyloxy)-9H-purin-2-ylamine, 6-(4-[^{123}I]iodo-benzyloxy)-9H-purin-2-ylamine, and 6-(4-[^{124}I]iodo-benzyloxy)-9H-purin-2-

ylamine.

24. The method of claim 13 wherein the mammal is a human.

25. The method of claim 13 wherein the radiolabeled alkyl or benzyl moiety comprises a radiolabel selected from the group consisting of ^{125}I, ^{123}I, ^{124}I, ^{131}I, and ^{18}F.

26. A composition useful for *in vivo* imaging of AGT molecules, comprising:

a radiolabeled O^6-benzylguanine molecule selected from the group consisting of 6-(4-[^{18}F]fluoro-benzyloxy)-9H-purin-2-ylamine, 6-(3-[^{131}I]iodo-benzyloxy)-9H-purin-2-ylamine, 6-(3-[^{125}I]iodo-benzyloxy)-9H-purin-2-ylamine, 6-(3-[^{123}I]iodo-benzyloxy)-9H-purin-2-ylamine, 6-(3-[^{124}I]iodo-benzyloxy)-9H-purin-2-ylamine, 6-(4-[^{18}F]fluoro-3-nitrobenzyloxy)-9H-purin-2-ylamine, 6-(4-[^{131}I]iodo-benzyloxy)-9H-purin-2-ylamine, 6-(4-[^{125}I]iodo-benzyloxy)-9H-purin-2-ylamine, 6-(4-[^{123}I]iodo-benzyloxy)-9H-purin-2-ylamine, and 6-(4-[^{124}I]iodo-benzyloxy)-9H-purin-2-ylamine; and

a pharmaceutically acceptable carrier.

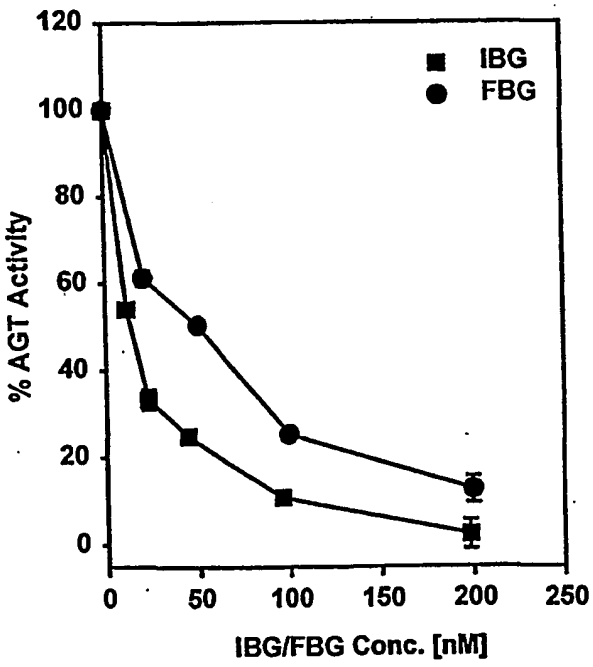

120
100
80
60
40
20
0
% AGT Activity
IBG
FBG
0
50
100
150
200
250
IBG/FBG Conc. [nM]

FIG. 1

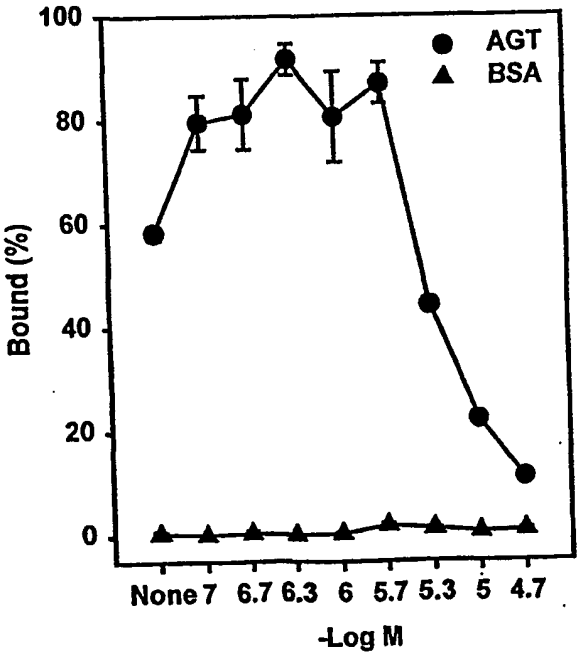


FIG. 2

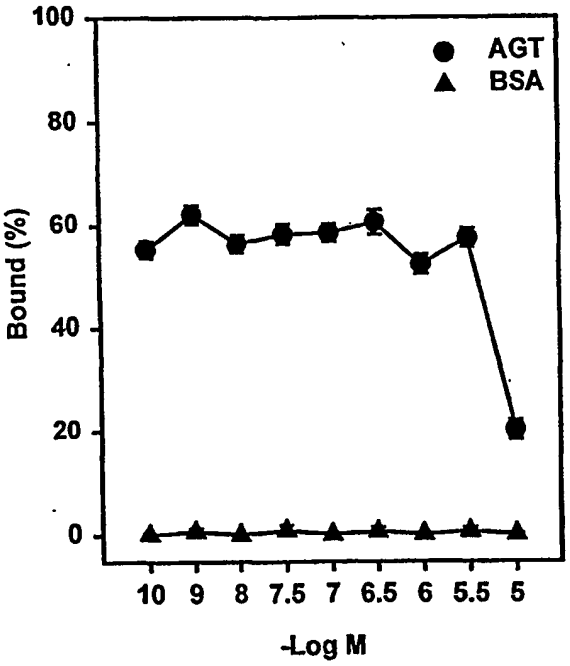
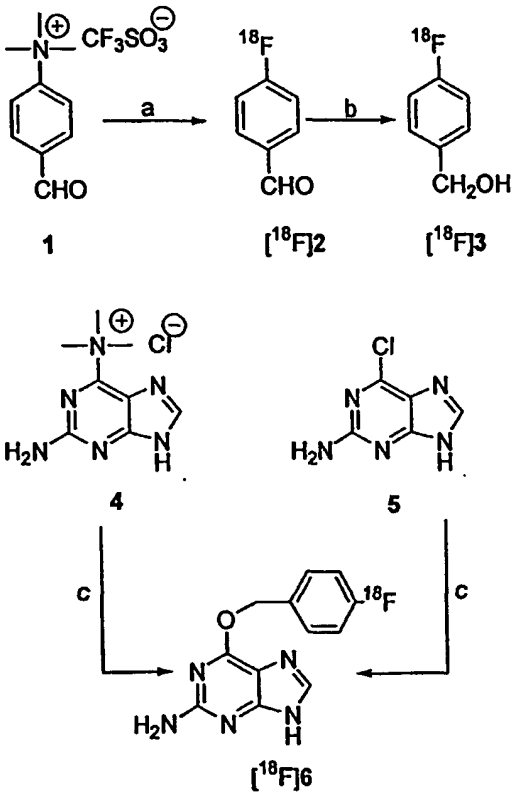


FIG. 3

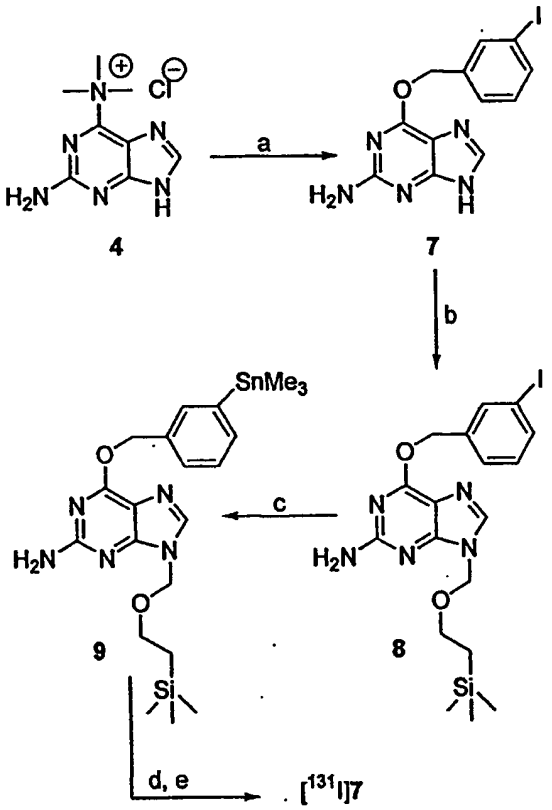
Scheme 1



(a) [^{18}F]Fluoride, DMSO; (b) DIBAL, CH_2Cl_2;
(c) **3**, Pot. *tert*-butoxide, DMSO

FIG. 4

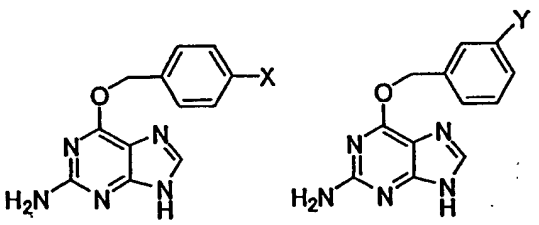
Scheme 2



(a) 3-Iodobenzyl alcohol, NaH, DMSO; (b) Pot. *tert*-butoxide, SEM-Cl, DMSO; (c) Hexamethylditin, $(Ph_3P)_2PdCl_2$, Dioxane; (d) I-131. H_2O_2, HOAc, sonication; (e) TFA or TBAF

FIG. 5

Chart I



X = F (6); Br (10); $SnMe_3$ (11); I (12)

Y = I (7); $SnMe_3$ (13)

FIG. 6